First-trimester Combined Test Screening for Down Syndrome: Results of a Series with 5,036 Cases

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SUMMARY

Objective: To assess the sensitivity of a first-trimester combined screening test for fetal chromosomal abnormalities.

Materials and Methods: From April 1999 to December 2002, 5,036 unselected women with singleton pregnancies underwent a first-trimester combined test (nuchal translucency thickness, pregnancy-associated plasma protein-A, and free β-human chorionic gonadotropin) screening for Down syndrome. They included 298 (5.9%) women aged 35 years or older and 4,738 (94.1%) women aged below 35 years. A positive result was defined as an estimated Down syndrome risk of greater than 1/270.

Results: Twenty-five (0.50%) chromosomal abnormalities were identified, with a false-positive rate of 7.1% (333/4,717) in women younger than 35 years and a false-positive rate of 17.3% (51/294) in women of advanced maternal age. The 25 chromosomal abnormalities included trisomy 21 in six fetuses, trisomy 18 and Turner's syndrome in four fetuses each, Klinefelter's syndrome, a marker chromosome and pseudohermaphroditism in one fetus each, and structural rearrangements in eight, including four with balanced translocations. One fetus with trisomy 18 was not discovered at screening, but a fetal ventricular septal defect was found on ultrasound at 30 weeks' gestation. The detection rate for fetal chromosomal abnormalities was 95.2% (20/21). If a cutoff of 1 in 200 was used, the false-positive rate would be 5.6% with the same detection rate, whereas the test still yielded a detection rate of 90.5% (19/21) with a 5% false-positive rate. In 83.3% of cases with trisomy 21 and 5.7% of normal pregnancies, fetal nuchal translucency thickness measurement was above the 95th percentile, whereas in 0.7% of the study population and in 24% of those with fetal chromosomal abnormalities, the fetal nuchal translucency thickness was at least 3 mm.

Conclusions: Our results indicate that the first-trimester combined test for Down syndrome is effective in identifying fetal chromosomal abnormalities. Moreover, the measurement of fetal nuchal translucency thickness at 10 to 13 weeks of gestation enables early detection of major fetal structural anomalies. [*Taiwanese J Obstet Gynecol* 2004;43(2):72–76]

Key Words: chromosomal abnormality, Down syndrome screening, human chorionic gonadotropin, nuchal translucency, pregnancy-associated plasma protein-A

Introduction

Down syndrome is the most commonly encountered chromosomal abnormality in newborns, with an incidence of 1 in 800 live births. In addition to decreased mentality, about half of cases have associated congenital heart disease. Down syndrome, therefore, has a substantial socioeconomic impact. A first-trimester combined test at 10 to 13 weeks' gestation is effective in detecting Down syndrome [1–9]. The test consists of measurements of sonographic fetal nuchal translucency (NT) thickness, and serum levels of pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (fβ-hCG). At a 5% false-positive rate, this test can detect Down syndrome in an additional 15% to 20% of cases compared with second-trimester biochemical screening [9–12].

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The combined test for Down syndrome first-trimester screening has been offered as an option to pregnant women at Cathay General Hospital since April 1999. This study analyzed the usefulness of the technique in detecting fetal chromosomal abnormalities.

Materials and Methods

From April 1999 to December 2002, a total of 5,036 unselected women with singleton pregnancies elected to have the combined test. The test involves measuring sonographic fetal NT thickness and maternal serum levels of fβ-hCG and PAPP-A at 10 to 13 weeks of gestation. Fetal NT thickness was measured according to the criteria of the Fetal Medicine Foundation in the UK [13]. Maternal serum levels of fβ-hCG and PAPP-A were determined within 1 week by microtiter-plate enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (Free β-hCG PNS EIA Kit and PAPP-A EIA Kit, Genemed Biotechnologies, South San Francisco, CA, USA). NT thickness, PAPP-A, and fβ-hCG values were divided by their respective day-specific medians to determine the multiples of the median (MoM) for each marker. Down syndrome risk was calculated using Alpha Software (Logical Medical Systems, London, UK) based on a multivariate Gaussian distribution as described by Wald et al [14]. A positive result was defined as an estimated Down syndrome risk of more than 1/270.

All chromosome analyses were carried out in the Cytogenetics Laboratory of Cathay General Hospital. For cultured chorionic villus tissue and amniocytes, four primary cultures were performed using an *in situ* method with CHANG MEDIUM® BMC (Irvine Scientific, Santa Ana, CA, USA). Microscopic analysis of Giemsa-stained chromosome banding used the rules for metaphase selection and colony definition as defined by Moertel et al [15].

Results

Among the 5,036 women, 298 (5.9%) were aged 35 years or older, and 4,738 (94.1%) were aged below 35 years. The median maternal age was 30.0 years, median maternal weight was 53.5 kg, and median gestational age at screening was 83 days. Table 1 shows the median values of NT thickness, PAPP-A, and fβ-hCG at 10 to 13 weeks of gestation. The median NT thickness was 1.0 mm, median PAPP-A was 3.5 mIU/mL, and median fβ-hCG was 50.0 mIU/mL. There were 406 positive-screen pregnancies which occurred in 7.5% (353/4,738) of women younger than 35 years and 17.8% (53/298) of women of advanced maternal age. Fetal NT thickness was above the 95th percentile in 83.3% of cases with trisomy 21 and in 5.7% of normal pregnancies, whereas it was at least 3 mm in 0.7% of all pregnancies and in 24% of those with fetal chromosomal abnormalities.

Table 1. Median levels of screening markers at 10–13 weeks of gestation

GA (wk)	NT (mm)	PAPP-A (mIU/mL)	fβ-hCG (mIU/mL)
10	0.8	2.1	65.0
11	0.9	2.9	53.0
12	1.0	4.2	46.0
13	1.0	5.4	37.0

GA = gestational age; NT = nuchal translucency thickness; PAPP-A = pregnancy-associated plasma protein-A; fβ-hCG = free beta-human chorionic gonadotropin.

Fetal karyotyping results were available for 309 (76%) of the 406 positive screening results. The other women were lost to follow-up, and no information regarding the outcome of their pregnancies was available; all pregnancies whose outcomes were unknown were assumed to be normal. Seven women underwent chorionic villus sampling in the first trimester, while the remaining 302 women underwent amniocentesis in the second trimester. Twenty-five chromosomal abnormalities were identified, including trisomy 21 in six fetuses, trisomy 18 in four, Turner's syndrome in four, Klinefelter's syndrome in one, a marker chromosome in one, pseudohermaphroditism in one, and structural rearrangements in eight, including four with balanced translocations. One fetus with trisomy 18 was not discovered at screening, but a ventricular septal defect was found by ultrasonography at 30 weeks' gestation.

Detailed screening results for the 25 cases with chromosomal abnormalities are shown in Table 2. A combination of a low PAPP-A level, a high fβ-hCG level, and increased NT thickness was noted in cases of trisomy 21 and monosomy X, whereas low levels of both PAPP-A and fβ-hCG were observed in cases of trisomy 18. Table 3 shows the detection and false-positive rates for fetal chromosomal abnormalities at different cutoff points. The same detection rate of 95.2% was achieved when the false-positive rate was lowered from 7.1% to 5.6%, which suggests that it would be reasonable to change the risk cutoff from the present 1 in 270 to 1 in 200. At a fixed 5% false-positive rate, the detection rate of the combined test in other studies ranged from 80% to 91% (Table 4) [1,3–5,7,9]. Table 5 illustrates the screening performance of the combined test for fetal

chromosomal abnormalities. The overall detection rate was 88.0%, with a false-positive rate of 7.7%.

Discussion

In this study, we demonstrated that in women younger than 35 years, the detection rate of fetal chromosomal abnormalities using the first-trimester combined test was 95.2% (at a risk cutoff of 1:270), with a false-positive rate of 7.1%. Even with a fixed 5% false-positive rate, the test still yielded a detection rate of 90.7% (at a risk cutoff of 1:140). As shown in Table 4, these results are in accordance with previous studies, which reported a detection rate in the range of 80% to 91% with a 5% false-positive rate. In addition to its higher detection rate, the combined test has also been shown to be more sophisticated and cost-effective than other second-trimester screening protocols [16–18]. However, in Taiwan, second-trimester maternal serum screening based

Table 2. Screening data for the 25 cases with fetal chromosomal abnormalities

MA (yr)	GA (d)	NT (MoM)	fβ-hCG (MoM)	PAPP-A (MoM)	DR	Fetal karyotype
37	81	1.37	1.49	0.6	1/240	46,XY,female
25	80	5.23*	1.08	0.53	1/3	45,X
37	93	2.02	0.59	0.2	1/16	47,XY,+18
31	89	2.2	2.67	0.37	1/16	47,XX,+21
22	90	0.71	3.13	0.13	1/90	45,X/47,XXX
31	84	0.55	0.25	0.48	1/29,000	47,XY,+18
32	90	8.86*	0.9	0.92	1/2	47,XX,+marker
26	80	9.78*	2.27	0.44	1/2	45,X
30	79	2.01	1.68	0.2	1/3	46,XY[21]/45,X[4]
30	86	2.14	1.55	0.23	1/4	47,XY,+21
34	76	14.2*	2.4	1	1/2	47,XY,+18
30	79	2.92	1.92	1.35	1/4	46,XY,t(6;16)(q14;q22)
34	72	2	1.22	1.21	1/150	46,XY,t(14;17)(q31;q24)pat
33	75	2.06	0.69	0.67	1/65	45,XX,der(13;14)(q10;q10)mat
33	82	3.14	1.11	0.63	1/2	47,XY,+21
32	84	1.76	0.61	0.43	1/120	47,XX,+21
30	97	1.28	4.77	0.48	1/85	45,XX,der(15;22)(q10;q10)
36	88	1.14	1.18	1.3	1/2,000	47,XXY
29	82	2.07	3.45	1.07	1/20	46,XX,ins(11;7)(p15.1;q31.1q21.2)
31	85	7.03*	0.19	0.39	1/2	47,XX,+18
30	86	2.91	2.88	0.89	1/2	47,XX,+21
29	82	1.96	1.44	0.39	1/18	46,X,del(X)(q23;q25)
29	84	2.81	0.66	1.02	1/9	46,XY,del(18)(p11.1)
32	80	2.05	1.97	0.3	1/3	47,XY,+21
36	82	1.17	0.62	0.68	1/1,700	46,XX,del(X)(p22.2;p21.2)

*MA = maternal age; GA = gestational age by ultrasound dating at the time of screening; NT = nuchal translucency thickness; fβ-hCG = free beta-human chorionic gonadotropin; PAPP-A = pregnancy-associated plasma protein-A; MoM = multiples of median; DR = Down syndrome risk. *NT ≥ 3 mm.*

Table 3. Observed detection rates and false-positive rates for fetal chromosomal abnormalities using different risk cutoffs

	Risk cutoff, %					
	1/140 (*n* = 237)		1/200 (*n* = 286)		1/270 (*n* = 353)	
	DR	FP	DR	FP	DR	FP
Total	90.5	5.0	95.2	5.6	95.2	7.1
T21	100.0	5.0	100.0	5.6	100.0	7.1
T18	75.0	5.0	75.0	5.6	75.0	7.1
45,X	100.0	5.0	100.0	5.6	100.0	7.1

n = number of positive screens in women aged below 35 years; DR = detection rate; FP = false-positive rate; T21 = trisomy 21; T18 = trisomy 18.

Table 4. Comparison of published studies giving detection and false-positive rates for trisomy 21 using the first-trimester combined test at various risk cutoffs

Authors	*n*	Markers	DR (%)	FP (%)	Risk cutoff
Spencer et al (1999) [1]	210	NT + fβ-hCG + PAPP-A	89.0	5.0	1/270
Wald et al (1999) [3]	77	NT + fβ-hCG + PAPP-A	85.0	5.0	1/250
Krantz et al (2000) [4]	50	NT + fβ-hCG + PAPP-A	91.0	5.0	1/270
Bindra et al (2002) [5]	82	NT + fβ-hCG + PAPP-A	90.2	5.0	1/215
Crossley et al (2002) [7]	81	NT + fβ-hCG + PAPP-A	82.0	5.0	1/250
Wald & Hackshaw (1997) [9]	163	NT + fβ-hCG + PAPP-A	80.0	5.0	1/390
Current study	6	NT + fβ-hCG + PAPP-A	100.0	5.0	1/140

All protocols include maternal age. n = number of cases; NT = nuchal translucency thickness; fβ-hCG = free beta-human chorionic gonadotropin; PAPP-A = pregnancy-associated plasma protein-A; DR = detection rate; FP = false-positive rate.

Table 5. Screening performance of the combined test for fetal chromosomal abnormalities

	Overall		Women < 35 yr		Women ≥ 35 yr	
	A	B	A	B	A	B
Screen positive, *n*	22	384	20	333	2	51
Screen negative, *n*	3	4,627	1	4,384	2	243
Total	25	5,011	21	4,717	4	294
Detection rate, %	88.0		95.2		50.0	
Specificity, %	92.3		92.9		82.7	
Positive predictive value, %	5.4		5.7		3.8	
Negative predictive value, %	99.9		100.0		99.2	
Prevalence, %	0.5		0.4		1.3	
False-positive rate, %	7.7		7.1		17.3	
Screen-positive rate, %	8.1		7.5		17.8	

A = number of affected cases; B = number of unaffected cases.

on the combination of maternal age, α-fetoprotein, and fβ-hCG remains the most widely used Down syndrome screening protocol. This yields a detection rate of only 56% to 70% for a false-positive rate of 5% [9–12,19]. Therefore, we encourage tertiary centers in Taiwan to implement the combined test as part of the clinical Down syndrome screening protocol.

The effectiveness of the combined test heavily depends on the accuracy of the sonographic measurement of fetal NT thickness. Standardization and quality control of fetal ultrasound NT examinations should therefore be emphasized [7]. Accurate measurement of NT thickness should be undertaken by fully qualified, well-trained sonographers who strictly follow the guidelines of the Fetal Medicine Foundation [13]. In this study, we found that in 83.3% of trisomy 21 cases and 5.7% of normal pregnancies, the fetal NT thickness measured was above the 95th percentile of the normal range. These results are similar to the rates of 73.7% and 4.8%, respectively, reported by von Kaisenberg et al [8]. Thus, ultrasonographic measurement of first-trimester NT thickness is feasible and useful in identifying Down syndrome. This screening method also identified 24% (6/25) of the fetal chromosomal abnormalities based on the finding of an NT thickness of at least 3 mm. However, fetal NT thickness alone is not an efficient screening marker for Down syndrome in the first trimester, because in order to achieve a detection rate of 85%, the false-positive rate would be as high as 21.1%. When combined with PAPP-A and fβ-hCG, the false-positive rate was only 6% with the same detection rate of 85% [12].

Deviations from the medians of the screening markers have been associated with adverse pregnancy outcomes [20–23]. Increased NT thickness is not only associated with congenital heart defects, chromosomal abnormalities, and fetal loss, but also with gestational hypertension and pre-eclampsia [22]. Low serum PAPP-A in the first trimester is also associated with fetal growth retardation, gestational hypertension, preterm delivery, and spontaneous miscarriage [23]. Therefore, in addition to detecting fetal aneuploidies, the combined test can identify women at increased risk of adverse pregnancy outcomes.

Recent reports have indicated that the integrated test will probably be the test of choice for high-

performance Down syndrome screening. The Serum, Urine and Ultrasound Screening Study showed that the integrated test, which includes NT thickness and five serum markers, yielded an 85% detection rate at a false-positive rate of 1.2%, and a 93% detection rate at a false-positive rate of 4.5% [12].

In women of advanced maternal age, the current standard of care is to offer an invasive prenatal diagnosis with amniocentesis or chorionic villus sampling. In this study, the detection rate in women of advanced maternal age was only 50% at a false-positive rate of 17.3%. We would not recommend the first-trimester combined test for women of advanced age; instead, the integrated test might be a better option. However, further study is required to address this issue so that the number of amniocentesis- and unnecessary procedure-related fetal losses can be reduced.

In conclusion, this study demonstrates that the combined test is an effective Down syndrome screening protocol in the first trimester. Furthermore, the sonographic measurement of fetal NT thickness at 10 to 13 weeks of gestation is a useful tool for the early diagnosis of fetal structural anomalies.

References

1. Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 1999;13:231–237.
2. Thilaganathan B. First-trimester nuchal translucency and maternal serum biochemical screening for Down's syndrome: a happy union? *Ultrasound Obstet Gynecol* 1999;13:229–230.
3. Wald NJ, Watt HC, Hackshaw AK. Integrated screening for Down's syndrome on the basis of tests performed during the first and second trimesters. *N Engl J Med* 1999;341:461–467.
4. Krantz DA, Hallahan TW, Orlandi F, Buchanan P, Larsen JW, Macri JN. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. *Obstet Gynecol* 2000;96:207–213.
5. Bindra R, Heath V, Liao A, Spencer, Nicolaides KH. One-stop clinic for assessment of risk for trisomy 21 at 11-14 weeks: a prospective study of 15,030 pregnancies. *Ultrasound Obstet Gynecol* 2002;20:219–225.
6. Schuchter K, Hafner E, Stangl G, Metzenbauer M, Hofinger D, Philipp K. The first trimester 'combined test' for the detection of Down syndrome pregnancies in 4939 unselected pregnancies. *Prenat Diagn* 2002;22:211–215.
7. Crossley JA, Aitken DA, Cameron AD, McBride E, Connor JM. Combined ultrasound and biochemical screening for Down's syndrome in the first trimester: a Scottish multicentre study. *Br J Obstet Gynaecol* 2002;109:667–676.
8. von Kaisenberg CS, Gasiorek-Wiens A, Bielicki M, et al. Screening for trisomy 21 by maternal age, fetal nuchal translucency and maternal serum biochemistry at 11-14 weeks: a German multicenter study. *J Matern Fetal Neonatal Med* 2002; 12:89–94.
9. Wald NJ, Hackshaw AK. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. *Prenat Diagn* 1997;17:821–829.
10. Spencer K. Second trimester prenatal screening for Down's syndrome using alpha-fetoprotein and free beta hCG: a seven year review. *Br J Obstet Gynaecol* 1999;106:1287–1293.
11. Nicolaides KH, Bindra R, Heath V, Cicero S. One-stop clinic for assessment of risk of chromosomal defects at 12 weeks of gestation. *J Matern Fetal Neonatal Med* 2002;12:9–18.
12. Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *Health Technol Assess* 2003;7:1–88.
13. Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet* 1998;352:343–346.
14. Wald NJ, Cuckle HS, Densem JW, et al. Maternal serum screening for Down's syndrome in early pregnancy. *BMJ* 1988;297:883–887.
15. Moertel CA, Stupca PJ, Dewald GW. Pseudomosaicism, true mosaicism, and maternal cell contamination in amniotic fluid processed with in situ culture and robotic harvesting. *Prenat Diagn* 1992;12:671–683.
16. Caughery AB, Kuppermann M, Norton ME, Washington AE. Nuchal translucency and first trimester biochemical markers for Down syndrome screening: a cost-effectiveness analysis. *Am J Obstet Gynecol* 2002;187:1239–1245.
17. Gilbert RE, Augood C, Gupta R, et al. Screening for Down's syndrome: effects, safety, and cost effectiveness of first and second trimester strategies. *BMJ* 2001;323:423–425.
18. Cusick W, Buchanan P, Hallahan TW, Krantz DA, Larsen JW, Macri JN. Combined first-trimester versus second-trimester serum screening for Down syndrome: a cost analysis. *Am J Obstet Gynecol* 2003;188:745–751.
19. Hsu JJ, Hsieh TT, Hsieh FJ. Down syndrome screening in an Asian population using alpha-fetoprotein and free beta-hCG: a report of the Taiwan Down Syndrome Screening Group. *Obstet Gynecol* 1996;87:943–947.
20. Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early pregnancy levels of pregnancy-associated plasma protein A and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. *J Clin Endocrinol Metab* 2002;87:1762–1767.
21. Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early-pregnancy origins of low birth weight. *Nature* 2002;417:916.
22. Tsai MS, Lee FK, Cheng CC, Hwa KY, Cheong ML, She BQ. Association between fetal nuchal translucency thickness in first trimester and subsequent gestational hypertension and preeclampsia. *Prenat Diagn* 2002;22:747–751.
23. Yaron Y, Heifetz S, Ochshorn Y, Lehavi O, Orr-Urtreger A. Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome. *Prenat Diagn* 2002;22:778–782.